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(11) **CA 2 240 609** (13) **A1**

(43) 14.10.1999

(12)

(21) 2 240 609

(51) Int. Cl.:

**C12N 005/10**, A01K 067/027,  
G01N 033/68, C12Q 001/68,  
C12N 015/85

(22) 11.08.1998

(30) 09/059,461 US 14.04.1998

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(54) METHODE DE CREATION D'ORGANISMES HYPERMUTABLES

(54) A METHOD FOR GENERATING HYPERMUTABLE ORGANISMS

(57)

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(51) Int.Cl.<sup>6</sup> C12N 5/10, C12N 15/85, C12Q 1/68, G01N 33/68, A01K 67/027  
(30) 1998/04/14 (09/059,461) US  
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**A METHOD FOR GENERATING HYPERMUTABLE ORGANISMS****ABSTRACT**

5        Dominant negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepared more efficiently than by relying on the natural rate of mutation.

## A METHOD FOR GENERATING HYPERMUTABLE ORGANISMS

This invention was made using a U.S. government grant from the NIH (CA43460). Therefore, the U.S. government retains certain rights to the invention.

### 5      TECHNICAL FIELD OF THE INVENTION

The invention is related to the area of mismatch repair genes. In particular it is related to the field of mutagenesis.

### BACKGROUND OF THE INVENTION

10      Within the past four years, the genetic cause of the Hereditary Nonpolyposis Colorectal Cancer Syndrome (HNPCC), also known as Lynch syndrome II, has been ascertained for the majority of kindreds affected with the disease (13). The molecular basis of HNPCC involves genetic instability resulting from defective mismatch repair (MMR). To date, six genes have been identified in humans that encode for proteins and appear to participate

15      in the MMR process, including the *mutS* homologs *GTBP*, *hMSH2*, and *hMSH3* and the *mutL* homologs *hMLH1*, *hPMS1*, and *hPMS2* (2,7,11,17,20,21,22, 24). Germline mutations in four of these genes (*hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2*) have been identified in HNPCC

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kindreds (2,11,13,17,24). Though the mutator defect that arises from the MMR deficiency can affect any DNA sequence, microsatellite sequences are particularly sensitive to MMR abnormalities (14). Microsatellite instability is therefore a useful indicator of defective MMR. In addition to its occurrence in virtually all tumors arising in HNPCC patients, Microsatellite instability is found in a small fraction of sporadic tumors with distinctive molecular and phenotypic properties (27).

HNPCC is inherited in an autosomal dominant fashion, so that the normal cells of affected family members contain one mutant allele of the relevant MMR gene (inherited from an affected parent) and one wild-type allele (inherited from the unaffected parent). During the early stages of tumor development, however, the wild-type allele is inactivated through a somatic mutation, leaving the cell with no functional MMR gene and resulting in a profound defect in MMR activity. Because a somatic mutation in addition to a germ-line mutation is required to generate defective MMR in the tumor cells, this mechanism is generally referred to as one involving "two hits," analogous to the biallelic inactivation of tumor suppressor genes that initiate other hereditary cancers (11,13,25). In line with this two-hit mechanism, the non-neoplastic cells of HNPCC patients generally retain near normal levels of MMR activity due to the presence of the wild-type allele.

#### **SUMMARY OF THE INVENTION**

It is an object of the present invention to provide a method for rendering cells hypermutable.

It is another object of the present invention to provide genetically altered cell lines.

It is yet another object of the present invention to provide a method to produce transgenic animals that are hypermutable.

It is also an object of the present invention to provide genetically altered transgenic animals.

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It is a further object of the invention to provide a method of mutating a gene of interest in a cell.

Yet another object of the invention is to provide a method of mutating a gene of interest in an animal.

5           These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention, a method for making a hypermutable cell is provided. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell. The cell becomes hypermutable as a result of  
10           the introduction of the gene.

          In another embodiment of the invention, an isolated hypermutable cell is provided. The cell comprises a dominant negative allele of a mismatch repair gene.

          In another embodiment of the invention, a hypermutable transgenic  
15           animal is provided. The animal comprises a dominant negative allele of a mismatch repair gene.

          In another embodiment of the invention, a method is provided for introducing a mutation into a gene of interest. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell.  
20           The cell becomes hypermutable as a result of the introduction of the gene. The cell further comprises a gene of interest. The cell is grown. The cell is tested to determine whether the gene of interest harbors a mutation.

          In another embodiment of the invention, a method is provided for generating a mutation in a gene of interest. A transgenic animal comprising  
25           a polynucleotide encoding a dominant negative allele of a mismatch repair gene is grown. The animal comprises a gene of interest. The animal is tested to determine whether the gene of interest harbors a mutation.

          These and other embodiments of the invention provide the art with methods that can generate enhanced mutability in cells and animals as well  
30           as providing cells and animals harboring potentially useful mutations.

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**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1. Diagrams of *PMS2* expression vectors (Fig. 1A) and pCAR reporters (Fig. 1B).

Figure 2. SH cells co-transfected with pCAR reporters and *PMS2* expression vectors after 17 days of drug selection. (Fig. 2A) Western blots of lysates from untransfected SH cells (lane 1) or SH cells transfected with *PMS2-NOT* (lane 2) or *PMS2-WT* (lane 3). The arrow indicates the 110 kD protein expected for hPMS2. (Fig. 2B) Western blots of lysates from untransfected SH cells (lane 1) or SH cells transfected with *PMS2-NOT* (lane 2) or *PMS2-134* (lane 3). The arrow indicates the 14 kD protein expected for hPMS-134. Both A and B were probed with an antibody generated against the N-terminus of hPMS2. The upper polypeptides in A and the lower polypeptides in B represent cross-reactive hamster proteins. (Fig. 2C)  $\beta$ -galactosidase activity in lysates derived from SH cells co-transfected with *PMS2-NOT* (lane 1), *PMS2-WT* (lane 2), or *PMS2-134* (lane 3) plus reporter plasmid. Relative  $\beta$ -galactosidase activities are defined as the ratio of  $\beta$ -galactosidase activity in cells transfected with pCAR-OF compared to that in cells transfected with pCAR-IF; this normalization controlled for transfection efficiency and controlled for  $\beta$ -galactosidase activity in the cells expressing the various *PMS2* effector genes.

Figure 3. *In situ*  $\beta$ -galactosidase activity of pooled clones of SH cells stably transduced with the *PMS2-NOT* (Fig. 3A), *PMS2-WT* (Fig. 3B), or *PMS2-134* (Fig. 3C) expression vectors, then re-transfected with pCAR-OF reporter. After 17 days of drug selection, the colonies were pooled, cultured, and stained for  $\beta$ -galactosidase activity. A pooled culture of of *PMS2-134* transduced SH cells expressing  $\beta$ -galactosidase from pCAR-OF

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is visible in Fig. 3C. The level of expression is lower, as expected, than in SH cells transfected with the pCAR-IF reporter plasmid, shown as a positive control in Fig. 3D. Each of the fields illustrated is representative of that found in triplicate experiments.

Figure 4. Protein expression and  $\beta$ -galactosidase activity in stably transduced SH clones. (Fig. 4A) Western blots of lysates from clones stably transduced with PMS2-NOT (lanes 1-3) or PMS2-WT (lanes 4-6). (Fig. 4B) Western blots of lysates from clones stably transduced with PMS2-NOT (lanes 1-3) or PMS2-134 (lanes 4-6). (The arrows indicate the polypeptide of the appropriate molecular weight. The upper (Fig. 4A) and lower (Fig. 4B) molecular weight polypeptides are nonspecific proteins. (Fig. 4C) The clones expressing PMS2-NOT (lane 1A-3A), PMS2-WT (lanes 1B-3B), or PMS2-134 (lanes 1C-3C) were transduced with pCAR-OF or pCAR-IF reporter plasmids and multiple subclones selected in hygromycin plus geneticin were harvested 17 days later and assayed for  $\beta$ -galactosidase activity. Relative  $\beta$ -galactosidase activities are defined as the ratio of  $\beta$ -galactosidase activity in cells transduced with pCAR-OF compared to that in cells transduced with pCAR-IF.

Figure 5. Immunoprecipitation of *in vitro* translated hPMS2 and hMLH1 proteins. (Fig. 5A) Labelled (indicated by an asterisk) or unlabelled proteins were incubated with an antibody to the C-terminus of hPMS2 in lanes 1-3 and to hMLH1 in lanes 4-6. Lane 7 contains a nonprogrammed reticulocyte lysate. The PMS-135 contains codons 135-862 of hPMS2. The major translation products of hPMS2 and hMLH1 are indicated. (Fig. 5B) Labelled hPMS-134 (containing codons 1-134 of hPMS2) was incubated in the presence or absence of unlabelled hMLH1 plus an antibody to hMLH1 (lanes 1 and 2, respectively). Lane 3 contains lysate from a nonprogrammed reticulocyte lysate. (Fig. 5C) Labelled proteins were incubated with an antibody to the N-terminus of hPMS2. Lane 6 contains a nonprogrammed



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reticulocyte lysate. In both Fig. 5A and Fig. 5B, autoradiographs of immunoprecipitated products are shown.

Figure 6. Complementation of MMR activity in transduced SH cells.

Lysates from pooled clones stably transduced with *PMS2-NOT*, *PMS2-WT*, or *PMS2-134* were complemented with purified *MutS $\alpha$*  or *MutL $\alpha$*  MMR components using the 5'G/T heteroduplex substrate. The values are presented as the percentage of repair activity in each case compared to that in lysates complemented with both purified *MutL $\alpha$*  and *MutS $\alpha$*  components to normalize for repair efficiency in the different lysate backgrounds. The values shown represent the average of at least three different determinations.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The inventors have discovered a method for developing hypermutable cells and animals by taking advantage of newly discovered alleles of human mismatch repair genes. Dominant negative alleles of such genes, when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable cells or animals can then be utilized to develop new mutations in a gene of interest.

The process of mismatch repair, also called mismatch proofreading, is carried out by protein complexes in cells ranging from bacteria to mammalian cells. A mismatch repair gene is a gene that encodes one of the proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, a mismatch repair complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base which is complementary to the older DNA strand. In this way, cells eliminate many mutations which occur as a result of mistakes in DNA replication.

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Dominant negative alleles cause a mismatch repair defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of a mismatch repair gene is the human gene *hPMS2-134*, which carries a truncation mutation at codon 134. The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele. Any allele which produces such effect can be used in this invention.

Dominant negative alleles of a mismatch repair gene can be obtained from the cells of humans, animals, yeast, bacteria, or other organisms. Such alleles can be identified by screening cells for defective mismatch repair activity. The cells may be mutagenized or not. Cells from animals or humans with cancer can be screened for defective mismatch repair. Cells from colon cancer patients may be particularly useful. Genomic DNA, cDNA, or mRNA from any cell encoding a mismatch repair protein can be analyzed for variations from the wild type sequence. Dominant negative alleles of a mismatch repair gene can also be created artificially, for example, by producing variants of the *hPMS2-134* allele or other mismatch repair genes. Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable cells or animals can be evaluated by testing the mismatch repair activity caused by the allele in the presence of one or more wild-type alleles, to determine if it is a dominant negative allele.

A cell or an animal into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or animals is elevated compared to cells or animals without such alleles. The degree of elevation

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of the spontaneous mutation rate can be at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal cell or animal.

According to one aspect of the invention, a polynucleotide encoding a dominant negative form of a mismatch repair protein is introduced into a cell or a transgenic animal. The gene can be any dominant negative allele encoding a protein which is part of a mismatch repair complex, for example, *PMS2*, *PMS1*, *MLH1*, or *MSH2*. The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of genomic DNA, cDNA, RNA, or a chemically synthesized polynucleotide. The polynucleotide can be introduced into the cell by transfection.

Transfection is any process whereby a polynucleotide is introduced into a cell. The process of transfection can be carried out in a living animal, e.g., using a vector for gene therapy, or it can be carried out *in vitro*, e.g., using a suspension of one or more isolated cells in culture. The cell can be any type of eukaryotic cell, including, for example, cells isolated from humans or other primates, mammals or other vertebrates, invertebrates, and single celled organisms such as protozoa or yeast.

In general, transfection will be carried out using a suspension of cells, or a single cell, but other methods can also be applied as long as a sufficient fraction of the treated cells or tissue incorporates the polynucleotide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably expressed in the cell. Techniques for transfection are well known. Available techniques for introducing polynucleotides include but are not limited to electroporation, transduction, cell fusion, the use of calcium chloride, and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been transfected with the mismatch repair gene, the cell can be grown and reproduced in culture. If the transfection is stable,

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such that the gene is expressed at a consistent level for many cell generations, then a cell line results.

An isolated cell is a cell obtained from a tissue of humans or animals by mechanically separating out individual cells and transferring them to a suitable cell culture medium, either with or without pretreatment of the tissue with enzymes, *e.g.*, collagenase or trypsin. Such isolated cells are typically cultured in the absence of other types of cells. Cells selected for the introduction of a dominant negative allele of a mismatch repair gene may be derived from a eukaryotic organism in the form of a primary cell culture or an immortalized cell line, or may be derived from suspensions of single-celled organisms.

A polynucleotide encoding a dominant negative form of a mismatch repair protein can be introduced into the genome of an animal by producing a transgenic animal. The animal can be any species for which suitable techniques are available to produce transgenic animals. For example, transgenic animals can be prepared from domestic livestock, *e.g.*, cows, pigs, sheep, goats, horses, etc.; from animals used for the production of recombinant proteins, *e.g.*, cows, pigs, or goats that express a recombinant protein in their milk; or experimental animals for research or product testing, *e.g.*, mice, rats, hamsters, guinea pigs, rabbits, etc.

Any method for making transgenic animals known in the art can be used. According to one process of producing a transgenic animal, the polynucleotide is injected into a fertilized egg of the animal and the injected egg is placed into a pseudo-pregnant female. The egg develops into a mature animal in which the polynucleotide is incorporated and expressed. The fertilized egg is produced *in vitro* from the egg and sperm of donor animals of the same species as the pseudo-pregnant female, who is prepared by hormone treatments to receive the fertilized egg and become pregnant. An alternative method for producing transgenic animals involves introducing the polynucleotide into embryonic cells by injection or transfection and reintroducing the embryonic cells into the developing embryo. With this

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method, however, if the polynucleotide is not incorporated into germline cells, the gene will not be passed on to the progeny. Therefore, a transgenic animal produced by this method must be evaluated to determine whether the gene is incorporated into germ cells of the animal. Once transgenic animals are produced, they can be grown to reproductive age, when they can be mated to produce and maintain a colony of transgenic animals.

Once a transfected cell line or a colony of transgenic animals has been produced, it can be used to generate new mutations in one or more gene(s) of interest. A gene of interest can be any gene naturally possessed by the cell line or transgenic animal or introduced into the cell line or transgenic animal. An advantage of using such cells or animals to induce mutations is that the cell or animal need not be exposed to mutagenic chemicals or radiation, which may have secondary harmful effects, both on the object of the exposure and on the workers.

Mutations can be detected by analyzing for alterations in the genotype of the cells or animals, for example by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. Mutations can also be detected by screening the phenotype of the gene. A mutant phenotype can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene. One can also screen for altered function of the protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the cell or animal associated with the function of the gene of interest.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

**EXAMPLE 1: *hPMS2-134* Encodes a Dominant Negative Mismatch Repair Protein.**

A profound defect in MMR was found in the normal cells of two HNPCC patients. That this defect was operative *in vivo* was demonstrated by the widespread presence of microsatellite instability in non-neoplastic cells of such patients. One of the two patients had a germ-line truncating mutation of the *hPMS2* gene at codon 134 (the *hPMS2-134* mutation), while the other patient had a small germ-line deletion within the *hMLH1* gene (26). These data thus contradicted the two-hit model generally believed to explain the biochemical and biological features of HNPCC patients. The basis for this MMR deficiency in the normal cells of these patients was unclear, and several potential explanations were offered. For example, it was possible that the second allele of the relevant MMR gene was inactivated in the germ-line of these patients through an undiscovered mechanism, or that unknown mutations of other genes involved in the MMR process were present that cooperated with the known germ-line mutation. It is clear from knock-out experiments in mice that MMR-deficiency is compatible with normal growth and development, supporting these possibilities (1,3,6). Alternatively, it was possible that the mutant alleles exerted a dominant negative effect, resulting in MMR deficiency even in the presence of the wild-type allele of the corresponding MMR gene and all other genes involved in the MMR process. To distinguish between these possibilities, we expressed the truncated polypeptide encoded by the *hPMS2-134* mutation in an MMR proficient cell line and analyzed its affect on the cell's MMR activity. The results showed that this mutant could indeed exert a dominant negative effect, resulting in biochemical and genetic manifestations of MMR deficiency.

The MMR proficient Syrian hamster TK<sup>+</sup>ts13 cell line (hereafter called SH cells) was cotransfected with various *hPMS2* expression plasmids plus reporter constructs for assessing MMR activity. The *hPMS2* expression plasmids contained the normal *hPMS2* gene product or the

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truncated *hPMS2* gene identified in the patient described above (*PMS2*-WT and *PMS2*-134, respectively, Fig. 1A). An "empty" vector devoid of *hPMS2* sequences (*PMS2*-NOT, Fig. 1A) served as an additional control. The reporter construct pCAR-OF (out of frame) contained a hygromycin resistance gene plus a  $\beta$ -galactosidase gene containing a 29 bp out-of-frame poly-CA tract at the 5' end of its coding region. The reporter construct pCAR-IF (in frame) was identical except that the poly-CA tract was 27 bp and therefore did not disrupt the  $\beta$ -galactosidase reading frame (Fig. 1B). The pCAR-OF reporter would not generate  $\beta$ -galactosidase activity unless a frame-restoring mutation (*i.e.*, insertion or deletion) arose following transfection.

Three different transfection schemes were used to evaluate the effects of the *PMS2*-134 mutation on SH cells. In the first scheme, the expression vectors plus the reporters were co-transfected together. Pools containing greater than 100 clones were generated following selection with hygromycin for 17 days and harvested for Western blot and  $\beta$ -galactosidase assays. SH cells transduced with *PMS2*-WT and *PMS2*-134 synthesized polypeptides of the expected size, as assessed with anti-hPMS2 antibodies on Western blots (Fig. 2A and 2B). As expected, virtually no  $\beta$ -galactosidase activity was observed in SH cells transfected with the pCAR-OF reporter plus *PMS2*-NOT (Fig. 2C). However, SH cells transfected with *PMS2*-134 expressed considerable  $\beta$ -galactosidase activity, significantly more than those transfected with *PMS2*-WT (Fig. 2C). These results suggested that the truncated polypeptide encoded by the *PMS2*-134 construct perturbs the endogenous MMR machinery, resulting in deletions or insertions that restored the reading frame. The exact nature of these presumed deletions or insertions could not be assessed, as multiple copies of the reporter constructs were transduced under our conditions, and the wild type  $\beta$ -galactosidase sequence was in great

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excess over the expected mutants, precluding their demonstration by direct sequencing.

In the second scheme, SH cells were co-transfected with each of the PMS2 expression vectors plus the hygromycin-resistance plasmid pLHL4. Hygromycin resistant cultures containing greater than 100 clones were pooled and expanded. These cultures were then co-transfected with pCAR-IF or pCAR-OF reporters plus a separate plasmid allowing geneticin selection. Two weeks later, the pooled cells, each containing more than 100 colonies resistant to both hygromycin and geneticin, were stained with X-gal to assess  $\beta$ -galactosidase activity. As shown in Figure 3, the cultures transfected with *PMS2-134* (panel C) contained many blue cells, while virtually no cells were blue in the cultures transfected with *PMS2-NOT* or *PMS2-WT* (panels A and B, respectively). In each case, transfection efficiency was controlled by parallel transfections using pCAR-IF which also served as a control for  $\beta$ -galactosidase activity of cells expressing the various PMS2 effector genes, which resulted in similar  $\beta$ -galactosidase expression levels in all cases (example in Fig. 3D). Increases in  $\beta$ -galactosidase activity after *PMS2-134* transfection compared to *PMS2-WT* transfection were also observed when a similar experimental protocol was applied to the MMR-proficient human embryonic kidney cell line 293. These cells were cotransfected with the pCAR-OF plus the various PMS2 effector plasmids and selected for 17 days in hygromycin. At day 17, colonies were stained with X-gal to assess  $\beta$ -galactosidase activity and scored for  $\beta$ -galactosidase expressing cells. As shown in Table 1, only those cells expressing the PMS2-134 polypeptide expressed a detectable  $\beta$ -galactosidase activity. These data demonstrate a similar dominant negative effect of the hPMS2-134 protein in both rodent and human systems and validate the utility of the rodent system in these studies.



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In the third scheme, SH cells were transfected with each of the *PMS2* expression vectors as described for the second scheme, but individual clones, rather than pooled clones, were expanded following drug selection. Of twenty clones transfected with *PMS2-WT*, five were shown to express readily detectable levels of full-length PMS2 proteins (examples in Fig. 4A, lanes 4-6). Similar analyses of twenty *PMS2-I34* clones revealed four clones which expressed truncated PMS2 polypeptides of the expected size (examples in Figure 4B, lanes 4-6). Three clones expressing full-length or truncated PMS2 proteins, as well as three randomly selected clones from *PMS2-NOT* transfected cells (Figure 4A and 4B, lanes 1-3) were chosen for further analysis. The individual clones were tested for  $\beta$ -galactosidase activity following co-transfection with pCAR-OF plus the pNTK plasmid, as described above for the pooled clones. As shown in Figure 4C, each of the three clones (lanes 3A-3C) expressing the truncated hPMS2 polypeptide yielded much higher  $\beta$ -galactosidase activities following transfection with pCAR-OF than did the clones expressing the full-length hPMS2 protein (lanes 2A-2C) or no hPMS2 protein (lanes 1A-1C).

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**Table 1.**  $\beta$ -galactosidase expression of 293 clones transfected with pCAR-OF reporter construct plus PMS2 effector plasmids. 293 cells were cotransfected with the pCAR-OF  $\beta$ -galactosidase reporter plasmid plus the *PMS2-NOT*, *-WT*, or *-I34* effector plasmids.

Transfected cells were selected in hygromycin for 17 days and stained with x-gal for  $\beta$ -galactosidase activity (blue colored cells). The results below represent the mean  $\pm$  standard deviation of triplicate experiments.

<u>Sample</u>	<u>Blue colonies</u>	<u>White colonies</u>
<i>PMS2-NOT</i>	0 $\pm$ 0	17 $\pm$ 2.7
<i>PMS2-WT</i>	0 $\pm$ 0	18 $\pm$ 4.0
<i>PMS2-I34</i>	15 $\pm$ 2.1	6 $\pm$ 2.1

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**Plasmids.** The full-length wild-type *hPMS2* cDNA was obtained from a human Hela cDNA library as described (18). An *hPMS2* cDNA containing a termination codon at amino acid 134 was obtained via RT-PCR from the patient in which the mutation was discovered (9). The cDNA fragments were cloned into the BamHI site into the pSG5 vector, which contains an SV40 promoter followed by an SV40 polyadenylation signal (8). The pCAR reporter vectors described in Fig. 1 were constructed as described in ref. 21 and 25.

**Cell lines and transfection.** Syrian Hamster fibroblast Tkts13 cells were obtained from ATCC and cultured as described (15). Stably transfected cell lines expressing *hPMS2* were created by cotransfection of the *PMS2* expression vectors and the pLHL4 plasmid encoding the hygromycin resistance gene at a ratio of 3:1 (pCAR:pLHL4) and selected with hygromycin. Stably transfected cell lines containing pCAR reporters were generated by co-transfection of pCAR vectors together with either pNTK plasmid encoding the neomycin resistance plasmid or with pLHL4. All transfections were performed using calcium phosphate as previously described (15).

**$\beta$ -galactosidase assay.** Seventeen days following transfection with pCAR,  $\beta$ -galactosidase assays were performed using 20  $\mu$ g of protein in 45 mM 2-mercaptoethanol, 1mM  $MgCl_2$ , 0.1 M  $NaPO_4$ , and 0.6 mg/ml Chlorophenol red- $\beta$ -D-galactopyranoside (CPRG, Boehringer Mannheim). Reactions were incubated for 1 hour, terminated by the addition of 0.5 M  $Na_2CO_3$ , and analyzed by spectrophotometry at 576 nm (16). For *in situ*  $\beta$ -galactosidase staining, cells were fixed in 1% glutaraldehyde in PBS and incubated in 0.15 M NaCl, 1 mM  $MgCl_2$ , 3.3 mM  $K_4Fe(CN)_6$ , 3.3 mM  $K_3Fe(CN)_6$ , 0.2% X-Gal for 2 hours at 37°C.

**EXAMPLE 2: hPMS2-134 Causes a Defect in MMR Activity**

The most likely explanation for the differences in  $\beta$ -galactosidase activity between *PMS2-WT* and *PMS2-134* transfected cells was that the *PMS2-134* protein disturbed MMR activity, resulting in a higher frequency of mutation within the pCAR-OF reporter and re-establishing the ORF. To directly test the hypothesis that MMR was altered, we employed a biochemical assay for MMR with the individual clones described in Fig. 4. Nuclear extracts were prepared from the clones and incubated with heteroduplex substrates containing either a /CA\ insertion-deletion or a G/T mismatch under conditions described previously. The /CA\ and G/T heteroduplexes were used to test repair from the 3' and 5' directions, respectively. There was a dramatic difference between the *PMS2-134* expressing clones and the other clones in these assays (Table 2A). While all clones repaired substrates from the 3' direction (/CA\ heteroduplex), cells expressing the *PMS2-134* polypeptide had very little 5' repair activity. A similar directional defect in mismatch repair was evident with pooled clones resulting from *PMS2-134* transfection, or when the heteroduplex contained a 2-4 base pair loop, examples of which are shown in Table 2B. A small decrease in MMR activity was observed in the 3' /CA\ *PMS2-WT* repair assays, perhaps a result of interference in the biochemical assays by overexpression of the *PMS2* protein. No significant activity was caused by *PMS2-WT* in the in situ  $\beta$ -galactosidase assays (Fig. 3; Table1), a result more likely to reflect the *in vivo* condition.

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**Table 2.** Mismatch repair activity of nuclear extracts from SH clones (A) or pooled cultures (B). The extracts were tested for MMR activity with 24 fmol of heteroduplex. \*These data represent similar results derived from greater than five independent experiments.

**A. SH clones\***

Repaired substrate (fmol/15 min)

3' /CA\      5' G/T

Cell Line

PMS2-NOT

clone A	10.2	3.5
clone B	12.7	2.9
clone C	13.5	5.5

PMS2-WT

clone A	2.8	2.2
clone B	5.7	4.8
clone C	4.7	2.9

PMS2-134

clone A	2.5	0.0
clone B	2.4	0.0
clone C	5.0	0.5

**B. Pooled cultures**

Repaired substrate (fmol/15 min)

3'G/T      5'G/T      3'/CTG\      5'/CTG\

Cell Line

PMS2-NOT	2.07 +/- 0.09	2.37 +/- 0.37	3.45 +/- 1.35	2.77 +/- 1.37
PMS2-WT	1.65 +/- 0.94	1.86 +/- 0.57	1.13 +/- 0.23	1.23 +/- 0.65
PMS2-134	0.14 +/- 0.2	0.0 +/- 0.0	1.31 +/- 0.66	0.0 +/- 0.0

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**Western blots.** Equal number of cells were lysed directly in lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.1 M 2-mercaptoethanol, 0.001% bromophenol blue) and boiled for 5 minutes. Lysate proteins were separated by electrophoresis on 4-12% Tris-glycine gels (for analysis of full-length hPMS2) or 4-20% Tris-glycine gels (for analysis of hPMS2-134). Gels were electroblotted onto Immobilon-P (Millipore) in 48 mM Tris base, 40 mM glycine, 0.0375% SDS, 20% methanol and blocked overnight at 4°C in Tris-buffered saline plus 0.05% Tween-20 and 5% condensed milk. Filters were probed with a polyclonal antibody generated against residues 2 - 20 of hPMS2 (Santa Cruz Biotechnology, Inc.) and a horseradish peroxidase conjugated goat anti-rabbit secondary antibody, using chemilluminescence for detection (Pierce).

***In vitro* translation.** Linear DNA fragments containing hPMS2 and hMLH1 cDNA sequences were prepared by PCR, incorporating sequences for *in vitro* transcription and translation in the sense primer. A full-length hMLH1 fragment was prepared using the sense primer 5'-ggatcctaatacagactcactatagggaga ccaccatgtcgttcgtggcaggg-3' (codons 1-6) and the antisense primer 5'-taagtcttaagtgtaccaac-3' (located in the 3' untranslated region, nt 2411-2433), using a wild-type hMLH1 cDNA clone as template. A full-length hPMS2 fragment was prepared with the sense primer 5'-ggatcctaatacagactcactatagggagaccaccatggaacaattgcctgcgg-3' (codons 1-6) and the antisense primer 5'-aggtagtgaagactctgtc-3' (located in 3' untranslated region, nt 2670-2690) using a cloned hPMS2 cDNA as template. A fragment encoding the amino-terminal 134 amino acids of hPMS2 was prepared using the same sense primer and the antisense primer 5'-agtcgaggtccaacctcg-3'. A fragment containing codons 135 - 862 of hPMS135 was generated using the sense primer 5'-ggatcctaatacagactcactatagggagaccaccatgatgtttgatcacaatgg-3' (codons 135-141) and the same antisense primer as that used for the full-length

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*hPMS2* protein. These fragments were used to produce proteins via the coupled transcription-translation system (Promega). The reactions were supplemented with  $^{35}\text{S}$ -labelled methionine or unlabelled methionine, as indicated in the text. The PMS135 and hMLH1 proteins could not be simultaneously radiolabelled and immunoprecipitated because of their similar molecular weights precluded resolution. Lower molecular weight bands are presumed to be degradation products and/or polypeptides translated from alternative internal methionines.

**Immunoprecipitation.** Immunoprecipitations were performed on *in vitro* translated proteins by mixing the translation reactions with 1  $\mu\text{g}$  of the MLH1 specific monoclonal antibody (mAb) MLH14 (Oncogene Science, Inc.), a polyclonal antibody generated to codons 2 - 20 of hPMS2 described above, or a polyclonal antibody generated to codons 843-862 of hPMS2 (Santa Cruz Biotechnology, Inc.) in 400  $\mu\text{l}$  of EBC buffer (50 mM Tris, pH 7.5, 0.1 M NaCl, 0.5% NP40). After incubation for 1 hr at 4°C, protein A sepharose (Sigma) was added to a final concentration of 10% and reactions were incubated at 4°C for 1 hour. Proteins bound to protein A were washed five times in EBC and separated by electrophoresis on 4-20% Tris-glycine gels, which were then dried and autoradiographed.

**Biochemical assays for mismatch repair.** MMR activity in nuclear extracts was performed as described, using 24 fmol of substrate (12,25). Complementation assays were done by adding ~ 100 ng of purified MutL $\alpha$  or MutS $\alpha$  components to 100  $\mu\text{g}$  of nuclear extract, adjusting the final KCl concentration to 100 mM (4,10,30). The substrates used in these experiments contain a strand break 181 nucleotides 5' or 125 nucleotides 3' to the mismatch. Values represent experiments performed at least in duplicate.

**EXAMPLE 3: Carboxy Terminus of hPMS2 Mediates Interaction between hPMS2 and hMLH1**

To elucidate the mechanism by which hPMS2-134 affected MMR, we analyzed the interaction between hPMS2 and hMLH1. Previous studies have shown that these two proteins dimerize to form a functionally active complex (12, 28). Proteins were synthesized *in vitro* using reticulocyte lysates, employing RNA generated from cloned templates. The full-length hMLH1 and hPMS2 proteins bound to each other and were co-precipitated with antibodies to either protein, as expected (data not shown). To determine the domain of hPMS2 which bound to hMLH1, the amino terminus (codons 1 - 134), containing the most highly conserved domain among mutL proteins (19,24), and the carboxyl terminus (codons 135 - 862) were separately cloned and proteins produced *in vitro* in coupled transcription-translation reactions. When a <sup>35</sup>S-labelled, full length hMLH1 protein (Fig. 5A, lane 5) was mixed with the unlabelled carboxyl terminal hPMS2 polypeptide, a monoclonal antibody (mAb) to the carboxyl terminus of hPMS2 efficiently immunoprecipitated the labeled hMLH1 protein (lane 1). No hMLH1 protein was precipitated in the absence of hPMS2 (lane 2). Conversely, when the <sup>35</sup>S-labelled carboxyl-terminus of hPMS2 (lane 3) was incubated with unlabelled, full-length hMLH1 protein, an anti-hMLH1 mAb precipitated the hPMS2 polypeptide (lane 4). In the absence of the unlabelled hMLH1 protein, no hPMS2 protein was precipitated by this mAb (lane 6). The same antibody failed to immunoprecipitate the amino-terminus of hPMS2 (amino acids 1-134) when mixed with unlabelled MLH1 protein (Fig. 5B, lane 1). This finding was corroborated by the converse experiment in which radiolabelled hPMS2-134 (Fig. 5C, lane 1) was unable to coprecipitate radiolabelled MLH1 when precipitations were done using an N-terminal hPMS2 antibody (Fig. 5C, lane 2) while this antibody was shown to be able to coprecipitate MLH1 when mixed with wild-type hPMS2 (Fig. 5C, lane 4).



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The initial steps of MMR are dependent on two protein complexes, called MutS $\alpha$  and MutL $\alpha$  (14). As the amino terminus of hPMS2 did not mediate binding of hPMS2 to hMLH1, it was of interest to determine whether it might instead mediate the interaction between the MutL $\alpha$  complex (composed of hMLH1 and hPMS2, ref. 12) and the MutS $\alpha$  complex (composed of MSH2 and GTBP, ref. 4). Because previous studies have demonstrated that MSH2 and the MutL $\alpha$  components do not associate in solution (28), we were unable to assay for direct hPMS2-134:MutS $\alpha$  interaction. We therefore used a different approach to address this issue, and attempted to complement nuclear extracts from the various SH cell lines with MutS $\alpha$  or MutL $\alpha$ . If the truncated protein present in the PMS2-134 expressing SH cells was binding to MutS $\alpha$  and lowering its effective concentration in the extract, then adding intact MutS $\alpha$  should rescue the MMR defect in such extracts. Purified MutS $\alpha$  added to such extracts had no effect (Fig. 6). In contrast, addition of intact MutL $\alpha$  to the extract completely restored directional repair to the extracts from PMS2-134 cells (Fig. 6).

The results described above lead to several conclusions. First, expression of the amino-terminus of hPMS2 results in an increase in microsatellite instability, consistent with a replication error (RER) phenotype. That this elevated microsatellite instability is due to MMR deficiency was proven by evaluation of extracts from stably transduced cells. Interestingly, the expression of PMS2-134 resulted in a polar defect in MMR, which was only observed using heteroduplexes designed to test repair from the 5' direction (no significant defect in repair from the 3' direction was observed in the same extracts). Interestingly, cells deficient in hMLH1 also have a polar defect in MMR, but in this case preferentially affecting repair from the 3' direction (5). It is known from previous studies in both prokaryotes and eukaryotes that the separate enzymatic components mediate repair from the two different directions. Our results, in combination with

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those of Drummond *et al.*, strongly suggest a model in which 5' repair is primarily dependent on hPMS2 while 3' repair is primarily dependent on hMLH1. It is easy to envision how the dimeric complex between PMS2 and MLH1 might set up this directionality. The combined results also demonstrate that a defect in directional MMR is sufficient to produce a RER+ phenotype.

We anticipated that the dominant negative function of the PMS2-134 polypeptide resulted from its binding to MLH1 and consequent inhibition of MutL $\alpha$  function. This hypothesis was based in part on the fact that the most highly conserved domain of the *PMS2* gene is located in its amino terminus, and the only known biochemical partner for PMS2 is MLH1. Our binding studies revealed, however, that the carboxyl terminus of PMS2, rather than the highly conserved amino terminus, actually mediated binding to MLH1. This result is consistent with those recently obtained in *S. cerevisiae*, in which the MLH1-interacting domain of PMS1 (the yeast homolog of human PMS2) was localized to its carboxyl-terminus (23). Our add-back experiments additionally showed that the hPMS2-134 mutant was not likely to mediate an interaction with the MutS $\alpha$  complex (Fig. 6). The best explanation at present to explain the various observations made here is that the hPMS2-134 polypeptide does not inhibit the initial steps in MMR, but rather interacts with and inhibits a downstream component of the pathway, perhaps a nuclease required for repair from the 5' direction.

The demonstration that the hPMS2-134 mutation can confer a dominant negative MMR defect to transfected cells helps to explain the phenotype of the kindred in which this mutant was discovered. Three individuals from this kindred were found to carry the mutation, a father and his two children. Both children exhibited microsatellite instability in their normal tissues and both developed tumors at an early age, while the father had no evidence of microsatellite instability in his normal cells and was completely healthy at age 35. The only difference known to us with respect to the MMR genes in this family is that the father's mutant allele was

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expressed at lower levels than the wild-type allele as assessed by sequencing of reverse transcriptase-PCR products of RNA from lymphocytes. The children expressed both alleles at approximately equal levels (Parsons *et al.* and unpublished observations). We suspect that the dominant negative attribute of the *hPMS2-134* mutant will only be manifest when it is present at sufficient concentrations (at least equimolar), thus explaining the absence of MMR deficiency in the father. The reason for the differential expression of the *hPMS2-134* allele in this kindred is not clear, though imprinting is a possibility. Hopefully, the ascertainment of additional, larger kindreds with such mutations will facilitate the investigation of this issue.

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## SEQUENCE LISTING

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- (iii) NUMBER OF SEQUENCES: 2
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(B) OPERATING SYSTEM: PC-DOS/MS-DOS  
(C) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: 2,240,609  
(B) FILING DATE: August 11, 1998  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER : US 09/059,461  
(B) FILING DATE: April 14, 1998  
(C) CLASSIFICATION:
- (viii) PATENT AGENT INFORMATION:  
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(B) REFERENCE NUMBER: JHW 19-1126

## (2) INFORMATION FOR SEQ. ID NO. 1.

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2771 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY : linear
- (ii) MOLECULAR TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 1
-

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ctt cgt cac aca aca gag aac aag cct cac agc cca aag act cca gaa	1347

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gca aag att tgt cct gga gaa aat caa gca gcc gaa gat gaa cta aga	2019
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aac tgt ccc cat gga agg cca acc atg aga cac atc gcc aac ctg ggt	2595
gtc att tct cag aac tgacgtagt cactgtatgg aataattggt tttatcgag	2650
atttttatgt ttgtaaagac agagtcttca ctaacctttt ttgttttaaa atgaaacctg	2710
ctacttaaaa aaaatacaca tcacacccat ttaaaagtga tcttgagaac cttttcaaac	2770
c	2771

## (2) INFORMATION FOR SEQUENCE ID. NO. 2

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 862 Amino Acids

(B) TYPE: amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE: Protein

(xi) SEQUENCE DESCRIPTION : SEQ. ID. NO. 2

```

Met Glu Arg Ala Glu Ser Ser Ser Thr Glu Pro Ala Lys Ala Ile Lys
 1           5           10           15
Pro Ile Asp Arg Lys Ser Val His Gln Ile Cys Ser Gly Gln Val Val
 20           25           30
Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp
 35           40           45
Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
 50           55           60
Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
 65           70           75           80
Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
 85           90           95
Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
100           105           110
Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Ala Ser
115           120           125
Ala Lys Val Gly Thr Arg Leu Met Phe Asp His Asn Gly Lys Ile Ile
130           135           140
Gln Lys Thr Pro Tyr Pro Arg Pro Arg Gly Thr Thr Val Ser Val Gln
145           150           155           160
Gln Leu Phe Ser Thr Leu Pro Val Arg His Lys Glu Phe Gln Arg Asn
165           170           175
Ile Lys Lys Glu Tyr Ala Lys Met Val Gln Val Leu His Ala Tyr Cys
180           185           190
Ile Ile Ser Ala Gly Ile Arg Val Ser Cys Thr Asn Gln Leu Gly Gln
195           200           205
Gly Lys Arg Gln Pro Val Val Cys Thr Gly Gly Ser Pro Ser Ile Lys
210           215           220
Glu Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile
225           230           235           240
Pro Phe Val Gln Leu Pro Pro Ser Asp Ser Val Cys Glu Glu Tyr Gly
245           250           255
Leu Ser Cys Ser Asp Ala Leu His Asn Leu Phe Tyr Ile Ser Gly Phe
260           265           270
Ile Ser Gln Cys Thr His Gly Val Gly Arg Ser Ser Thr Asp Arg Gln
275           280           285
Phe Phe Phe Ile Asn Arg Arg Pro Cys Asp Pro Ala Lys Val Cys Arg
290           295           300
Leu Val Asn Glu Val Tyr His Met Tyr Asn Arg His Gln Tyr Pro Phe
305           310           315           320
Val Val Leu Asn Ile Ser Val Asp Ser Glu Cys Val Asp Ile Asn Val
325           330           335

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Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu  
 340 345 350  
 Ala Val Leu Lys Thr Ser Leu Ile Gly Met Phe Asp Ser Asp Val Asn  
 355 360 365  
 Lys Leu Asn Val Ser Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu  
 370 375 380  
 Ile Lys Met His Ala Ala Asp Leu Glu Lys Pro Met Val Glu Lys Gln  
 385 390 395 400  
 Asp Gln Ser Pro Ser Leu Arg Thr Gly Glu Glu Lys Lys Asp Val Ser  
 405 410 415  
 Ile Ser Arg Leu Arg Glu Ala Phe Ser Leu Arg His Thr Thr Glu Asn  
 420 425 430  
 Lys Pro His Ser Pro Lys Thr Pro Glu Pro Arg Arg Ser Pro Leu Gly  
 435 440 445  
 Gln Lys Arg Gly Met Leu Ser Ser Ser Thr Ser Gly Ala Ile Ser Asp  
 450 455 460  
 Lys Gly Val Leu Arg Pro Gln Lys Glu Ala Val Ser Ser His Gly  
 465 470 475 480  
 Pro Ser Asp Pro Thr Asp Arg Ala Glu Val Glu Lys Asp Ser Gly His  
 485 490 495  
 Gly Ser Thr Ser Val Asp Ser Glu Gly Phe Ser Ile Pro Asp Thr Gly  
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 Ser His Cys Ser Ser Glu Tyr Ala Ala Ser Ser Pro Gly Asp Arg Gly  
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 Ser Gln Glu His Val Asp Ser Gln Glu Lys Ala Pro Glu Thr Asp Asp  
 530 535 540  
 Ser Phe Ser Asp Val Asp Cys His Ser Asn Gln Glu Asp Thr Gly Cys  
 545 550 555 560  
 Lys Phe Arg Val Leu Pro Gln Pro Thr Asn Leu Ala Thr Pro Asn Thr  
 565 570 575  
 Lys Arg Phe Lys Lys Glu Glu Ile Leu Ser Ser Ser Asp Ile Cys Gln  
 580 585 590  
 Lys Leu Val Asn Thr Gln Asp Met Ser Ala Ser Gln Val Asp Val Ala  
 595 600 605  
 Val Lys Ile Asn Lys Lys Val Val Pro Leu Asp Phe Ser Met Ser Ser  
 610 615 620  
 Leu Ala Lys Arg Ile Lys Gln Leu His His Glu Ala Gln Gln Ser Glu  
 625 630 635 640  
 Gly Glu Gln Asn Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu  
 645 650 655  
 Asn Gln Ala Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Thr Met  
 660 665 670  
 Phe Ala Glu Met Glu Ile Ile Gly Gln Phe Asn Leu Gly Phe Ile Ile  
 675 680 685  
 Thr Lys Leu Asn Glu Asp Ile Phe Ile Val Asp Gln His Ala Thr Asp  
 690 695 700  
 Glu Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly  
 705 710 715 720  
 Gln Arg Leu Ile Ala Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu  
 725 730 735  
 Ala Val Leu Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp  
 740 745 750  
 Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala Lys Leu Ile  
 755 760 765  
 Ser Leu Pro Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Val Asp  
 770 775 780  
 Glu Leu Ile Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro

785		790		795		800
Ser Arg Val Lys	Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val					
	805		810		815	
Met Ile Gly Thr Ala Leu Asn Thr Ser Glu Met Lys Lys Leu Ile Thr						
	820		825		830	
His Met Gly Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro						
	835		840		845	
Thr Met Arg His Ile Ala Asn Leu Gly Val Ile Ser Gln Asn						
850		855		860		

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## WE CLAIM:

1. A method for making a hypermutable cell, comprising the step of:  
introducing into a mammalian cell a polynucleotide comprising a  
5 dominant negative allele of a mismatch repair gene, whereby the cell  
becomes hypermutable.
2. The method of claim 1 wherein the polynucleotide is introduced by  
transfection of a suspension of cells *in vitro*.
3. The method of claim 1 wherein the mismatch repair gene is *PMS2*.
- 10 4. The method of claim 1 wherein the mismatch repair gene is human *PMS2*.
5. The method of claim 1 wherein the mismatch repair gene is human  
*MLH1*.
6. The method of claim 1 wherein the mismatch repair gene is human *PMS1*.
7. The method of claim 1 wherein the mismatch repair gene is human  
15 *MSH2*.
8. The method of claim 4 wherein the allele comprises a truncation  
mutation.
9. The method of claim 4 wherein the allele comprises a truncation mutation  
at codon 134 as shown in SEQ ID NO: 1.
- 20 10. The method of claim 9 wherein the truncation mutation is a thymidine at  
nucleotide 424 of wild-type *PMS2* as shown in SEQ ID NO: 1.
11. The method of claim 1 wherein the polynucleotide is introduced into a  
fertilized egg of an animal.
12. The method of claim 11 wherein the fertilized egg is subsequently  
25 implanted into a pseudo-pregnant female whereby the fertilized egg develops  
into a mature transgenic animal.
13. The method of claim 12 wherein the mismatch repair gene is *PMS2*.
14. The method of claim 12 wherein the mismatch repair gene is human  
*PMS2*.
- 30 15. The method of claim 12 wherein the mismatch repair gene is human  
*MLH1*.

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16. The method of claim 12 wherein the mismatch repair gene is human *PMS1*.
17. The method of claim 12 wherein the mismatch repair gene is human *MSH2*.
- 5 18. The method of claim 14 wherein the allele comprises a truncation mutation.
19. The method of claim 14 wherein the allele comprises a truncation mutation at codon 134 as shown in SEQ ID NO: 1.
20. The method of claim 19 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2* as shown in SEQ ID NO: 1.
- 10 21. A homogeneous composition of cultured, hypermutable, mammalian cells which comprise a dominant negative allele of a mismatch repair gene.
22. The isolated hypermutable cell of claim 21 wherein the mismatch repair gene is *PMS2*.
- 15 23. The isolated hypermutable cell of claim 21 wherein the mismatch repair gene is human *PMS2*.
24. The isolated hypermutable cell of claim 21 wherein the mismatch repair gene is human *MLH1*.
25. The isolated hypermutable cell of claim 21 wherein the mismatch repair gene is human *PMS1*.
- 20 26. The isolated hypermutable cell of claim 21 wherein the mismatch repair gene is human *MSH2*.
27. The isolated hypermutable cell of claim 21 wherein the cells express a protein consisting of the first 133 amino acids of hPMS2.
- 25 28. A hypermutable transgenic mammal wherein at least 50% of the cells of the mammal comprise a dominant negative allele of a mismatch repair gene.
29. The hypermutable transgenic animal of claim 28 comprising a protein which consists of the first 133 amino acids of human PMS2.
30. A method for generating a mutation in a gene of interest comprising the steps of:
- 30



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growing a mammalian cell comprising the gene of interest and a dominant negative allele of a mismatch repair gene, wherein the cell is hypermutable;

testing the cell to determine whether the gene of interest harbors a mutation.

31. The method of claim 30 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

32. The method of claim 30 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

33. The method of claim 30 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

34. The method of claim 30 wherein the step of testing comprises analyzing the phenotype of the gene of interest.

35. The method of claim 30 wherein the mammalian cell is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a mammalian cell, whereby the cell becomes hypermutable.

36. The method of claim 35 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

37. The method of claim 35 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

38. The method of claim 35 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

39. The method of claim 35 wherein the step of testing comprises analyzing the phenotype of the gene of interest.

40. A method for generating a mutation in a gene of interest comprising the steps of:

growing a mammal comprising the gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene;

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testing the mammal to determine whether the gene of interest harbors a mutation.

41. The method of claim 40 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

5 42. The method of claim 40 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

43. The method of claim 40 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

10 44. The method of claim 40 wherein the step of testing comprises analyzing the phenotype of the gene of interest.

45. The method of claim 40 wherein the mammal is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a mammal, whereby the mammal becomes hypermutable.

15 46. The method of claim 45 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

47. The method of claim 45 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

20 48. The method of claim 45 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

49. The method of claim 45 wherein the step of testing comprises analyzing the phenotype of the gene of interest.

50. A hypermutable transgenic mammal made by the method of claim 45.

51. The transgenic mammal of claim 50 wherein the mammal is a primate.

25 52. The transgenic mammal of claim 50 wherein the mismatch repair gene is *PMS2*.

53. The transgenic mammal of claim 50 wherein the mismatch repair gene is human *PMS2*.

30 54. The transgenic mammal of claim 50 wherein the mismatch repair gene is human *MLH1*.

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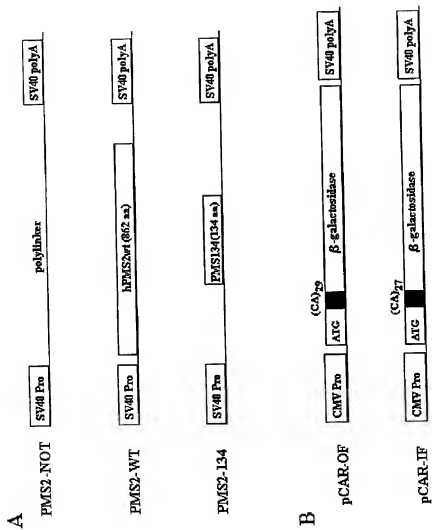
55. The transgenic mammal of claim 50 wherein the mismatch repair gene is human *PMS1*.

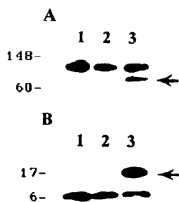
56. The transgenic mammal of claim 50 wherein the mismatch repair gene is human *MSH2*.

5 57. The transgenic mammal of claim 50 wherein the allele comprises a truncation mutation.

58. The transgenic mammal of claim 50 wherein the allele comprises a truncation mutation at codon 134 as shown in SEQ ID NO: 1.

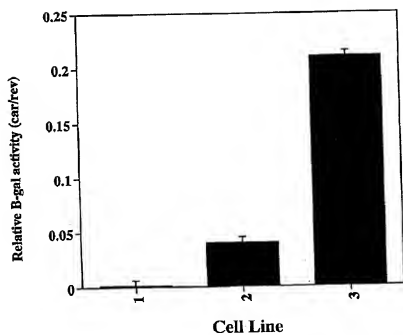
10 59. The transgenic mammal of claim 58 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2* as shown in SEQ ID NO: 1.





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Figure 2

**C**



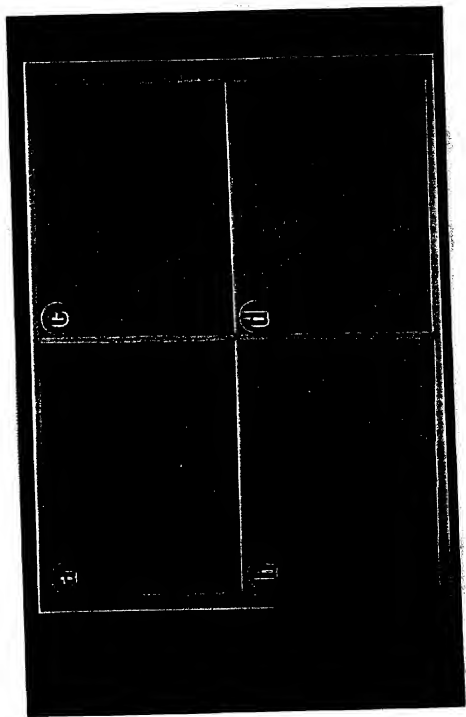


Fig 3

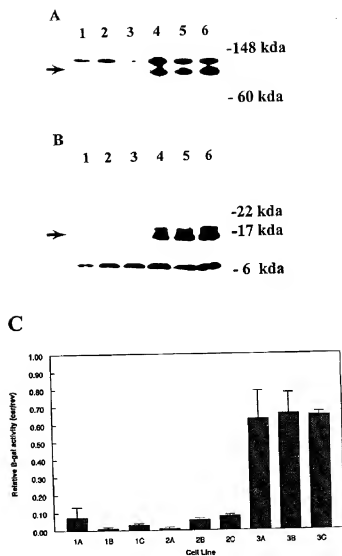
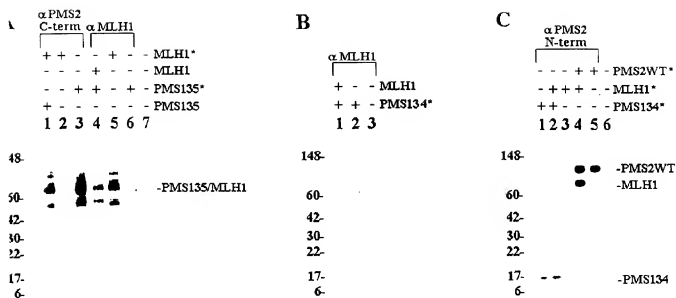


Fig 4





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Figure 6